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Effects of Phenol, 2,4-Dimethylphenol 2,4-Dichlorophenol, and Pentachlorophenol on Embryo, Larval, and Early-Juvenile Fathead Minnows ('Pimephales promelas')

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Effects of Phenol, 2,4-Dimethylphenol, 2,4-Dichlorophenol, and Pentachlorophenol on Embryo, Larval, and Early-Juvenile Fathead Minnows (*Pimephales promelas*)

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Abstract. Embryos of fathead minnows were more resistant to phenol, 2,4-dimethylphenol (2,4-DMP), 2,4-dichlorophenol (2,4-DCP), and pentachlorophenol (PCP) than were larval or juvenile life stages. Growth of 28-day-old fish was the most sensitive indicator of stress during exposures to phenol, 2,4-DMP, and PCP, whereas survival was the most sensitive indicator of toxic effects from 2,4-DCP exposure. Based on these effects, the estimated maximum acceptable toxicant concentration for fathead minnows in Lake Superior water lies between 1,830 and 3,570 μg/L for phenol; 1,970 and 3,110 μg/L for 2,4-DMP; 290 and 460 μg/L for 2,4-DCP; and 44.9 and 73.0 μg/L for PCP.

Phenolic wastes are common water pollutants generated from a variety of industrial processes such as those used in oil refineries, gas operations, coke ovens, coal gasification, and by natural processes such as the decomposition of plant matter. Phenol, 2,4-dimethylphenol (2,4-DMP), and 2,4-dichlorophenol (2,4-DCP) are widely used in chemical manufacturing operations as basic chemical feedstocks. Phenol is used in the production of phenolic resins, bis-phenol, the synthesis of halogenated and alkylated phenols, and in some plastics and medicines. The compound 2,4-DMP is used as a feedstock for producing phenolic antioxidants, disinfectants, solvents, pharmaceuticals, insecticides, fungicides, plasticizers, rubber chemicals, polyphenylene oxide, dyestuffs, wetting agents, and as an additive or constituent of gasolines, lubricants, and cresylic acid. The compound 2,4-DCP is used in the manufacture of herbicides, germicides, temporary soil sterilants, seed disinfectants, plant-growth regulators, mothproofing agents, miticides, and wood preservatives. Pentachlorophenol (PCP) is used mainly as a wood preservative, but it is also used in adhesives, leather, photographic solutions, petroleum drilling mud, rope, burlap, and sealing gaskets, in water cooling towers, and in secondary oil recovery. An estimated 80 million pounds of PCP were produced in the United States in 1977 (Moore 1979). The widespread uses of the phenolic compounds and their presence in some aquatic environments dictate a need for information on the long-term effects of these compounds on aquatic organisms.

An extensive literature review revealed no information on the long-term toxicity of phenol or 2,4-DCP to any freshwater fish species, although one life-cycle test with the cladoceran *Daphnia magna* has been conducted with phenol (U.S. Environmental Protection Agency 1978). No data were found dealing with long-term effects of PCP on fathead minnows, but several investigators (Crandall and Goodnight 1962; Brockway 1963; Webb and Brett 1973) have studied the effects of sublethal concentrations of PCP on several other species of freshwater fish. Data from one fathead minnow embryo-larval test were reported with 2,4-DMP by the U.S. Environmental Protection Agency (1978). Very few acute toxicity data exist for 2,4-DCP and 2,4-DMP with aquatic organisms, although acute data are abundant for phenol and PCP with both fish and invertebrates. Acute data, however, are of limited value for estimating long-term effects of these compounds on aquatic life.

The purpose of this study was to determine the toxicity of phenol, 2,4-DMP, 2,4-DCP, and PCP by conducting 32-day flow-through tests with the embryo-larval and early-juvenile life stages of the fathead minnow. McKim (1977) concluded that tests with the embryo-larval and early-juvenile life stages could, in most cases, be used to estimate the maximum acceptable toxicant concentration (MATC) for a compound within a factor of two. Information on embryo and larval survival and larval growth derived from the present study was used

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to estimate the MATC for each tested phenolic compound.

Materials and Methods

Exposure System

The exposure system consisted of a modified proportional diluter (Mount and Brungs 1967) with a dilution factor of 0.6. Every diluter cycle delivered 2 L of water to flow-splitting chambers (Benoit and Puglisi 1973), which divided each of six concentrations four ways into quadruplicate exposure tanks. The glass exposure tanks measured 50.0 × 14.5 × 15.0 cm deep with a 10.0-cm standpipe, which yielded a tank volume of 7.3 L. Flow rates averaged 53 ml/min, and replacement time for 90% of the test water was 5.0 hr as determined by the method of Sprague (1969). All tanks were scrubbed and siphoned once or twice per week depending on biological growth in the tanks. Except for short periods of time when dead eggs were removed each day, embryos were not subjected to direct light. A constant photoperiod of 16 hr was used during all larval exposures, and fluorescent lamps were used as the source of illumination. The light intensity measured 48 lumens at the water surface during larval and early-juvenile portions of the tests.

Water Characteristics

Water in all tests was obtained from Lake Superior. Temperatures were maintained at a constant 25° ± 2° C throughout the tests by a Honeywell temperature controller.[1,2] Total hardness, alkalinity, acidity, pH, and dissolved oxygen were determined from one series of quadruplicate tanks once per week. The means and ranges for total hardness, alkalinity, and acidity were 46.0 (44.6–47.0), 41.9 (40.2–44.0), and 1.9 (1.0–3.2) mg/L as $CaCO_3$, respectively, during the four tests. The pH range during the four tests was 7.2 to 7.9, and the mean dissolved oxygen was 7.7 mg/L (range 6.5 to 8.5). Chemical measurements were made with procedures described by the American Public Health Association (1975).

Toxicant Solutions

Reagent grade phenol, 2,4-DMP, 2,4-DCP, and PCP were obtained from either the Eastman Kodak Company[3] or from the Matheson, Coleman and Bell Company.[4] The compounds were dissolved in distilled water and introduced to the diluter by a chemical metering device (Mount and Warner 1965). Water samples were taken from the quadruplicate tanks so that toxicant concentrations were measured in all tanks once a week. Water samples for phenol, 2,4-DMP, and 2,4-DCP were analyzed with the automated 3-methyl-2-benzothiazolone hydrazone (MBTH) method (Friestad *et al.* 1969) as modified by Gales (1975). The distillation step was unnecessary and was eliminated. Samples were diluted with distilled water, and the concentrations were calculated on the basis of a standard curve for distilled water standards. The compound PCP was analyzed by gas chromatography with a Hewlett-Packard[5] model 5730A gas chromatograph. The method was taken from the Pierce Handbook (Pierce Chemical Company 1977–1978) and involved forming a derivative of pentachlorophenol by using the reagent Tri-Sil BSA (Formula D).[6] Mean recoveries and standard deviations for spiked samples were: phenol, 89% ± 15% (N = 24); 2,4-DMP, 103% ± 7% (N = 10); 2,4-DCP, 103% ± 9% (N = 13); and PCP, 90% ± 14% (N = 18). Mean measured concentrations were relative standard deviations for these compounds are given in Tables 1 to 4.

Biological Procedures

Embryos were obtained from the stock culture unit maintained at the Environmental Research Laboratory-Duluth. Tests were begun by distributing, at random, groups of 50 eggs (<24 hr after spawning) to each of the 24 exposure tanks. Eggs were incubated in oscillating cups made from 0.12-L glass jars with 40-mesh nylon screen bottoms. With the use of a dissecting microscope, all nonviable eggs were removed from the cup and discarded on the day after the beginning of each test. Dead embryos were removed daily thereafter until hatching was complete. At complete hatch, the number of normal larvae was recorded, and 25 normal larvae from each cup were placed in their respective tanks for larval exposure. Larvae that were dead, obviously deformed, or suffering from severe pericardial edema were considered abnormal. Equal amounts of live San Francisco Bay brine shrimp (<12 hr old) were fed to the fish in all tanks three times a day Monday through Friday and once a day on weekends, throughout the tests. Surviving fish were counted at 96 hr, 1 week, 2 weeks, 3 weeks, and 4 weeks. Remaining fish were killed in ice water 4 weeks after hatch for final weight measurements.

Statistical Analyses

Percentage hatchability and percentage survival data were normalized by using an arcsin square root transformation procedure described by Bartlett (1947). Normalized hatchability and survival data and growth data were subjected to one-way analysis of variance with an F-test of significance (P = 0.05) and to subsequent analysis with Dunnett's one-sided comparison of treatment means to control means (P = 0.05) (Steel and Torrie 1960). Growth data of individual fish from each series of quadruplicate tanks were combined before conducting the analysis of variance.

Results

Phenol

Growth of 28-day-old fish was significantly reduced at a phenol concentration of 3,570 μg/L (Table 1). Survival of fish at the end of the test was not significantly affected at any phenol concentration tested. Based on growth effects, the estimated MATC for

[1] The U.S. Environmental Protection Agency neither recommends nor endorses any commerical product; trade names are used only for identification.

[2] Honeywell, Inc., 1100 Virginia Drive, Fort Washington, PA 19034.

[3] Eastman Kodak Co., Rochester, NY 14650.

[4] Matheson, Coleman and Bell Co., Norwood, OH 45212.

[5] Hewlett-Packard, Route 41, Avondale, PA 19311.

[6] Available from the Pierce Chemical Co., Rockford, IL 61105.

Table 1. Survival and growth of fathead minnows exposed to various concentrations of phenol. Asterisk (*) denotes values significantly less than controls (analysis of variance and Dunnett's procedure, P = 0.05)

Measured concentration (μg/L)	Percentage normal larvae at hatch at each concentration	Percentage survival at each concentration	Average weight of all fish exposed at each concentration (mg)
Control			
Not detectable	88.4 ± 3.2[a,b]	79.0 ± 7.6[a,c]	103.0 ± 22.4[a]
240 ± 150[a]	91.5 ± 5.7	75.0 ± 8.2	99.6 ± 24.5
450 ± 240	92.5 ± 5.5	74.0 ± 12.0	101.5 ± 22.4
910 ± 360	88.5 ± 1.0	73.0 ± 17.1	101.4 ± 26.5
1,830 ± 480	90.5 ± 6.6	74.0 ± 20.3	98.9 ± 24.5
3,570 ± 660	93.0 ± 2.6	87.0 ± 3.8	84.8 ± 20.0*

[a] Mean ± standard deviation of the combined quadruplicate tanks
[b] Two hundred eggs exposed per concentration
[c] Survival based on 100 larvae per concentration

phenol and fathead minnows lies between 1,830 and 3,570 μg/L.

2,4-DMP

Exposure of fathead minnows to 2,4-DMP caused significant reductions in both survival and growth. Survival was significantly reduced by exposure to 5,130 μg/L, but growth, a more sensitive indicator of effect, was significantly reduced at both 3,110 and 5,130 μg/L (Table 2). The estimated MATC for 2,4-DMP and fathead minnows, based on growth effects, lies between 1,970 and 3,110 μg/L.

2,4-DCP

Survival of fathead minnows was significantly reduced after 28 days of exposure to 2,4-DCP concentrations of 460 μg/L and above; growth was significantly reduced at 1,240 μg/L (Table 3). The estimated MATC for 2,4-DCP and fatheads, based on survival, lies between 290 and 460 μg/L.

PCP

Pentachlorophenol was the only compound tested that caused a significant reduction in the percentage of normal larvae at hatch (Table 4). The percentage of normal larvae at hatch was slightly lower in all PCP concentrations and in the controls than in the other three tests because of a higher number of nonviable eggs in the batch of embryos used to start the PCP test. All larvae that hatched and survived at 223 μg PCP/L, however, were lethargic and had severe pericardial edema. The majority of these larvae would not respond to vibration or probing stimulus, and close examination with a microscope for the presence of a heartbeat was the only means of distinguishing between live and dead larvae. Only 2 of 100 larvae were alive 96 hr after they were released into PCP exposure tanks containing 223 μg/L, and none were alive after one week of exposure. Percentage survival of 28-day-old fish was significantly reduced by exposure to PCP concentrations of 128 and 228 μg/L. The growth of fathead minnows after 28 days was significantly reduced by PCP concentrations of 73.0 μg/L and above. Based on these growth effects, the estimated MATC for fathead minnows and PCP lies between 44.9 and 73.0 μg/L.

Discussion

PCP was the most toxic of the four compounds tested and was followed in order of decreasing toxicity by 2,4-DCP, 2,4-DMP, and phenol. The 96-hr LC50 values for PCP, 2,4-DCP, 2,4-DMP, and phenol were determined to be 221, 8,230, 16,800, and 28,800 μg/L, respectively, for fathead minnows in Lake Superior water (Phipps *et al.* 1981). Both acute and embryo-larval tests resulted in the same order of toxicity. Embryo-larval tests were much more sensitive, however, and significant adverse effects were observed at concentrations of PCP, 2,4-DCP, 2,4-DMP, and phenol that were 3.0, 17.9, 5.4, and 8.1 times lower than their respective 96-hr LC50 values. In the present tests, embryos were more resistant to phenolic compounds than were larval or early-juvenile life stages of fathead minnows. Effects observed on normal embryo hatch at the high PCP exposure (223 μg/L) were not surprising since this concentration was slightly higher than the 96-hr LC50 for juvenile fathead minnows (Phipps *et al.* 1981). The concentration of PCP that

Table 2. Survival and growth of fathead minnows exposed to various concentrations of 2,4-dimethylphenol. Asterisk (*) denotes values significantly less than controls (analysis of variance and Dunnett's procedure, P = 0.05)

Measured concentration (μg/L)	Percentage normal larvae at hatch at each concentration	Percentage survival at each concentration	Average weight of all fish exposed at each concentration (mg)
Control			
Not detectable	82.5 ± 1.9[a,b]	90.0 ± 9.5[a,c]	72.6 ± 26.5[a]
900 ± 110[a]	88.5 ± 4.1	78.0 ± 7.7	76.7 ± 22.4
1,360 ± 120	87.5 ± 3.0	78.0 ± 12.4	75.5 ± 20.0
1,970 ± 150	87.5 ± 3.0	75.0 ± 15.1	71.1 ± 22.4
3,110 ± 240	87.7 ± 5.3	73.0 ± 7.6	61.3 ± 22.4*
5,130 ± 150	79.9 ± 5.0	56.0 ± 16.7*	50.4 ± 17.3*

[a] Mean ± standard deviation of the combined quadruplicate tank
[b] Two hundred eggs exposed per concentration
[c] Survival based on 100 larvae per concentration

Table 3. Survival and growth of fathead minnows exposed to various concentrations of 2,4-dichlorophenol. Asterisk (*) denotes values significantly less than controls (analysis of variance and Dunnett's procedure, P = 0.05)

Measured concentration (μg/L)	Percentage normal larvae at hatch at each concentration	Percentage survival at each concentration	Average weight of all fish exposed at each concentration (mg)
Control			
Not detectable	81.5 ± 9.4[a,b]	75.0 ± 16.8[a,c]	91.9 ± 31.6[a]
150 ± 38[a]	85.9 ± 7.2	69.0 ± 26.0	94.1 ± 28.3
290 ± 43	82.1 ± 4.8	70.0 ± 14.8	91.6 ± 26.5
460 ± 43	76.0 ± 11.4	42.0 ± 12.0*	106.0 ± 37.4
770 ± 70	79.5 ± 6.8	22.0 ± 8.3*	84.6 ± 30.0
1,240 ± 97	80.2 ± 13.4	6.0 ± 5.2*	22.0 ± 10.0*

[a] Mean ± standard deviation of the combined quadruplicate tanks
[b] Two hundred eggs exposed per concentration
[c] Survival based on 100 larvae per concentration

affected embryo hatchability was more than three times higher than that which significantly reduced growth of 28-day-old fish. Normal embryo hatch was not affected at the highest exposure for 2,4-DMP, 2,4-DCP, and phenol during these tests. Preliminary testing with similar methods, however, indicated that 2,4-DMP and 2,4-DCP significantly affected normal hatch at 24,100 and 3,800 μg/L, respectively, whereas phenol concentrations equivalent to the 96-hr LC50 (Phipps *et al.* 1981) did not significantly affect normal hatch. Brockway (1963) found that the embryo stage of the cichlid fish *Cichlasoma bimaculatum* was also more resistant to PCP than were subsequent life stages.

Growth of 28-day old fish was the most sensitive indicator of stress during exposures with phenol, 2,4-DMP, and PCP, whereas survival was the most sensitive indicator of toxic effects woth 2,4-DCP. Both PCP and 2,4-DMP significantly affected growth at one concentration higher than those that significantly reduced survival, but only the highest phenol exposure reduced growth. Survival was significantly reduced at two concentrations of 2,4-DCP below that which caused significant reductions in growth. The differences in effect endpoints between 2,4-DCP and the other three compounds may indicate differences in mechanisms of toxic action. Survival effects were apparent much earlier in the tests than were growth effects. Reduction in survival of fathead minnows exposed to 2,4-DCP was readily apparent after 96 hr of larval exposure, and nearly all deaths had occurred within two weeks. Effects on survival were signinficant at 460 and 770 μg 2,4-DCP/L, although the fish that survived were apparently able to function and grow normally.

The estimated MATC for 2,4-DMP (1,970-3, 110 μg/L) from the present study compares very well with the estimated 2,4-DMP MATC (1,500–3,200 μg/L) derived for fathead minnows during previous embryo-larval testing (U.S. Environmental Protec-

Table 4. Survival and growth of fathead minnows exposed to various concentrations of pentachlorophenol. Asterisk (*) denotes values significantly less than controls (analysis of variance and Dunnett's procedure, P = 0.05)

Measured concentration (μg/L)	Percentage normal larvae at hatch at each concentration	Percentage survival at each concentration	Average weight of all fish exposed at each concentration (mg)
Control			
Not detectable	63.5 ± 7.0[a,b]	94.0 ± 2.3[a,c]	131.2 ± 26.5[a]
27.2 ± 2.7[a]	63.5 ± 6.0	92.0 ± 3.3	135.6 ± 26.5
44.9 ± 3.3	67.5 ± 10.6	92.0 ± 8.6	127.9 ± 33.2
73.0 ± 5.2	59.5 ± 8.2	87.0 ± 8.9	113.1 ± 31.6*
128.0 ± 8.5	63.0 ± 6.8	21.0 ± 11.9*	108.1 ± 36.1*
223.0 ± 17.4	0 ± 0*	0 ± 0*	—

[a] Mean ± standard deviation of the combined quadruplicate tanks
[b] Two hundred eggs exposed per concentration
[c] Survival based on 100 larvae per concentration

tion Agency 1978). No information was found concerning the long-term effects of either 2,4-DCP or phenol on any species of freshwater fish. In a life-cycle test with the cladoceran *Daphnia magna* (U.S. Environmental Protection Agency 1978), the MATC for phenol was 1,500–6,300 μg/L. The estimated MATC for phenol in the present study was 1,830–3,570 μg/L. Fatheads and daphnids appear to have similar sensitivity to phenol, but differences in the dilution factors used in these tests make it impossible to determine which was the most sensitive. The estimated MATC for PCP in the present study, based on growth effects, was 44.9–73.0 μg/L. Crandall and Goodnight (1962) also found significant reductions in the growth of the common guppy (*Lebistes reticulatus*) exposed to PCP concentrations of 500 μg/L. Webb and Brett (1973) determined that both growth rate and foodconversion efficiency of underyearling sockeye salmon (*Oncorhynchus nerka*) were reduced by PCP exposure to 3.49 μg/L and above. Exposure of steelhead trout (*Salmo gairdneri*) alevins to sodium pentachlorophenate (Na-PCP) increased oxygen-consumption rates, reduced yolk-utilization efficiency, and reduced growth (Chapman and Shumway 1978). They also found that continuous exposure to Na-PCP from fertilization to complete yolk-sac absorption produced 100% mortality 40 μg/L, but low mortality at 10 μg/L. Results of the two previously mentioned studies indicate that salmonid species may be more sensitive to PCP than are fathead minnows. The estimated MATC for PCP (47-88 μg/L) from a saltwater test (Parrish *et al.* 1972) with sheepshead minnows (*Cyprinodon variegatus*) compared well with the MATC from the present study. The MATC for sheepshead minnows was based on survival, however, and no effects were observed on growth or hatching success at the MATC concentrations.

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